July 2015. Horticultural Plant Journal, 1 (1): 41–47.

Horticultural Plant Journal

Available online at www.sciencedirect.com
The journal's homepage: http://www.journals.elsevier.com/horticultural-plant-journal

Allelic Tests and Sequence Analysis of Three Genes for Resistance to *Xanthomonas perforans* Race T3 in Tomato

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Received 31 May 2015; Received in revised form 18 June 2015; Accepted 26 June 2015

Abstract

Three crosses, Hawaii7981 × PI128216, Hawaii7981 × LA1589, and PI128216 × LA1589, were made to develop F_2 populations for testing allelism among three genes *Xv3*, *Rx4*, and Rx_{LA1589} conferring resistance to bacterial spot caused by *Xanthomonas perforans* race T3 in tomato. Each population consisted of 535–1655 individuals. An infiltration method was used to inoculate the leaves of the parental and F_2 plants as well as the susceptible control OH88119 for detecting hypersensitive resistance (HR). The results showed that all the tomato plants except OH88119 had HR to race T3, indicating that *Xv3*, *Rx4*, and Rx_{LA1589} were allelic genes. Genomic DNA fragments of the *Rx4* alleles from Hawaii7981, PI128216, and LA1589 were amplified using gene-specific primers and sequenced. No sequence variation was observed in the coding region of *Rx4* in the three resistant lines. Based on the published map positions of these loci as well as the allelic tests and sequence data obtained in this study, we speculated that *Xv3*, *Rx4*, and Rx_{LA1589} were the same gene. The results will provide useful information for understanding the mechanism of resistance to race T3 and developing resistant tomato varieties.

Keywords: tomato; tomato bacterial spot; race T3; hypersensitive resistance gene; allelic test

1. Introduction

Bacterial spot caused by the dominant *Xanthomonas perforans* race T3 is a bacterial disease of tomato in China (Sun et al., 1999; Yang, 2013). In the past decade as the planting pattern changes in China, it has gradually become one of the major diseases in protected tomato production areas (Wang et al., 2005; Guo et al., 2008; Liu et al., 2008; Zhang et al., 2010), and severely threatens the continuous development of the tomato industry.

Since the first occurrence of race T3 reported in the US state of Florida in 1995 (Jones et al., 1995), great efforts have been made to screen resistant plant sources, study the genetics, and map resistance to race T3. Several tomato lines including *Solanum lycopersicum* var. *cerasiforme* accession PI114490, *S. pimpinellifolium* accession PI340905-S, and *S. lycopersicum* lines PI126428 and PI155372 with field resistance to race T3 have been reported. Meanwhile, some lines including *S. pimpinellifolium* accessions PI126932, PI128216, and LA1589, *S. pennelli* accession LA716, and *S. lycopersicum* Hawaii7981 with both field resistance and hypersensitive response (HR) have also been identified (Scott et al., 1995; Astua-Monge et al., 2000; Sun et al., 2011a). Further studies suggested that HR was conditioned mainly by single dominant genes, while field resistance was inherited quantitatively (Scott et al., 1996, 2001;

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http://dx.doi.org/10.16420/j.issn.2095-9885.2015-0001

Robbins et al., 2009; Sun et al., 2011a, 2011b). Three genes, *Xv3*, *Rx4*, and Rx_{LA1589}, which confer HR to race T3, have been reported to date. These genes have been mapped to the same region of tomato chromosome 11, although they are from different tomato lines Hawaii7981, PI128216, and LA1589 (Sun et al., 2011a; Wang et al., 2011; Pei et al., 2012). Based on the map positions and allelic tests using a small number of individuals in segregating populations, *Xv3* and *Rx4* were found to be possibly allelic or the same gene (Wang et al., 2011; Yang, 2013).

To determine the relationship among *Xv3*, *Rx4*, and Rx_{LA1589}, crosses were made among three tomato lines Hawaii7981, PI128216, and LA1589 that carry these genes to develop F_2 populations. Allelic tests were conducted by disease evaluation in the F_2 populations. The sequences of the *Rx4* loci also were obtained through PCR amplification and sequencing. Whether *Xv3*, *Rx4*, and Rx_{LA1589} are the same gene was determined by sequence comparison. The results obtained here will provide useful information for understanding the mechanism of resistance to bacterial spot and the use of the three resistant lines in tomato breeding programs.

2. Materials and methods

2.1. Plant materials and experimental design

Three tomato lines with resistance to bacterial spot race T3 and their progenies were used in the study. Unimproved breeding line *S. lycopersicum* Hawaii7981 carries the *Xv3* gene. *S. pimpinellifolium* accessions PI128216 and LA1589 carry the *Rx4* and Rx_{LA1589} genes, respectively. Three crosses, Hawaii7981 × PI128216, Hawaii7981 × LA1589, and PI128216 × LA1589, were made to develop F_1 and F_2 populations. Two independent experiments for disease evaluation were conducted in a greenhouse at Shangzhuang Experimental Station of China Agricultural University (Beijing, China). The first experiment consisted of Hawaii7981, PI128216, and their F_1 and F_2 populations. Seeds were sown on 11 February 2014. The seedlings were transplanted on 21 March 2014 and inoculated with bacterial suspension of race T3 strain on 10 April 2014. The second experiment consisted of Hawaii7981, PI128216, and LA1589 as well as F_1 and F_2 populations of Hawaii7981 × LA1589 and PI128216 × LA1589. Seeds were sowed on 28 March 2014. The seedlings were transplanted on 26 April 2014, and inoculated with the bacterial suspension of race T3 strain on 18 May 2014. Elite tomato breeding line OH88119 was used as the susceptible control in both experiments.

2.2. Inoculation and observation of hypersensitive response

X. perforans race T3 strain *Xv*829 was obtained from the University of Florida (Dr. J. B. Jones). The bacteria were grown in Petri plates on yeast, dextrose, and calcium carbonate agar medium (Lelliot and Stead, 1987) at 28 ℃ for 2 to 3 days. Bacteria were washed from the agar with sterile double-distilled water (ddH_2O) and the suspension was adjusted to approximately 1×10^8 colony forming units (CFU) per mL.

One hour prior to inoculation, the plants were misted with water. Inoculations were performed by leaf infiltration (Yang and Francis, 2005) on three leaflets per plant. The bacterial suspension was infiltrated through the abaxial side of fully expanded leaflets using a 5 mL syringe without a needle until the infiltration area reached approximately 1 cm^2. The inoculated plants were kept in a protected greenhouse and misted with water twice per day (9:00 am and 5:00 pm) after infiltration to increase humidity for disease development. The HR response was inspected visually 24 hours after inoculation (HAI). The observation was maintained every 24 hours until water-soaked symptoms were observed on leaves of the susceptible control OH88119.

2.3. Amplification and analysis of Rx4 gene sequences

Genomic DNA was isolated from young leaves of Hawaii7981, PI128216, LA1589, and OH88119 using the modified CTAB method (Kabelka et al., 2002). The genomic DNA was used to amplify the DNA fragment of *Rx4* as well as up- and downstream sequences with a pair of gene-specific primers (Forward: 5′-TATTATCGGCAGGAAGCAC-3′, Reverse: 5′-CTTTCTTCTACAACGCCTC-3′) designed using the candidate *Rx4* sequence (Pei et al., 2012) and the tomato genome sequence (Sato et al., 2012).

PCR amplification was conducted in a 25 μL reaction volume consisting of 2.5 μL 10 × LA PCR Buffer II (Mg^{2+} Plus), 1 μL (10 μmol · L^{-1}) each primer, 4 μL dNTPs (2.5 μmol · L^{-1}), 2 μL DNA template, 1.25 units of TaKaRa LA *Taq* DNA polymerase (TaKaRa Biotechnology, Dalian, China), and 14.25 μL ddH_2O. Reactions were heated at 94 ℃ for 4 min followed by 34 cycles of 35 s at 94 ℃, 35 s at 52 ℃, and 5 min extension at 72 ℃. Final reactions were extended at 72 ℃ for 10 min. Amplification was performed in a BIO-RAD programmable thermal controller (Beijing, China).

The PCR products were separated on 1% agarose gel, purified using TIANgel Midi Purification Kit (TIANGEN Biotech, Beijing, China), and cloned into a pMD19-T vector (TaKaRa). Recombinants were verified by PCR, and at least two positive clones for each tomato line were sequenced at Sunbiotech Company (Beijing, China). The vector sequence was removed and the sequences for each tomato line were assembled using DNAMAN (Lynnon Biosoft, USA). The corresponding sequences of the *Rx4* locus for Heinz1706 and LA1589 were obtained from the SOL Genomics Networks (http://www.